
PYRROLIZIDINE ALKALOIDS
AND PHARMACOPHAGOUS
LEPIDOPTERA VISITORS OF
PRESTONIA AMABILIS
(APOCYNACEAE) IN A MONTANE
RAINFOREST IN ECUADOR¹

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ABSTRACT

Four species of Ithomiinae butterflies (Lepidoptera: Nymphalidae) were observed in nature taking up pyrrolizidine alkaloids (PAs) from withered flowers of *Prestonia amabilis* J. F. Morales (Apocynaceae, Echiteae) in a montane rainforest in southern Ecuador (ca. 1000 m a.s.l.). Quantitative experiments were subsequently carried out using either withered flowers or crushed roots of *P. amabilis*. Field trials were conducted in November 2000 at six locations in the area of the Reserva Biológica San Francisco (1800–2000 m a.s.l., ca. 15 km from the first site) where *P. amabilis* was not known to occur. A total of 40 specimens of 10 species of the Ithomiinae butterflies and 40 specimens of eight species of Arctiidae moths were quantitatively sampled. While the first group showed a clear preference for baits with withered flowers, the latter preferred the crushed roots. In total, 13 species of Ithomiinae were observed visiting PA sources. Eight of these 13 species have no previous records of pharmacophagy for PAs. Within the Ithomiinae, there is evidence of phylogenetic difference in attraction, with a noticeably high proportion of recorded species belonging to the Napeogenini. Analysis of plant material by gas chromatography–mass spectrometry revealed the presence of two novel retronecine monoesters of the lycopsamine type, tentatively identified as 5'-demethyllycopsamine (ideamine A) and 5'-demethylsolycopsamine or one of its diastereoisomers. The highest PA levels were found in flowers of *P. amabilis* (0.13%, dry weight basis), followed by roots (0.075%) and leaves (0.044%). In flowers, ideamine A accounted for 84% of total PAs, whereas roots contained ideamine A (53%) and 32% of its 3'-acetyl ester. We suggest that *Prestonia* R. Br. may have served as an ancestral source of PAs in the evolution of pharmacophagous behavior in the Ithomiinae.

Key words: Apocynaceae (Echiteae), Arctiidae, chemical defense, Ctenuchini, Euchromiini, Ithomiinae, *Prestonia*, pyrrolizidine alkaloid.

Plants have developed a large array of secondary substances for protection against herbivores, while the herbivores have found various ways to overcome these chemical defenses. One of the most remarkable phenomena that has resulted from this evolutionary arms race is the ability of certain herbivores not only to cope with the plant's secondary defensive compounds, but even to sequester and use these substances for their own benefit (e.g., Nishida,

2002). The pyrrolizidine alkaloids (PAs) are one of the best-studied examples. The ability of insects to sequester PAs is phylogenetically widespread. Examples include chrysomeline beetles (Ehmke et al., 1999; Hartmann et al., 1999; Pasteels et al., 2003), ithomiine and danaine butterflies, and arctiid moths. Insect species obtaining PAs from plants avoid accumulation of the toxic PA free base (Hartmann & Ober, 2000). Adapted Lepidoptera convert the PA free

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base into its nontoxic *N*-oxide (Lindigkeit et al., 1997; Brückmann et al., 2000; Naumann et al., 2002), whereas the *N*-oxide is easily reduced in the gut of unspecialized herbivores and predators. PAs are taken up either with eaten plant material or independently from nutrition, the latter behavior termed pharmacophagy (Boppré, 1984).

This paper examines the pharmacophagous behavior in two Lepidoptera taxa, the Ithomiinae (Nymphalidae) and the Ctenuchini–Euchromiini clade (Arctiidae, Arctiinae), visiting PA-containing parts of *Prestonia* R. Br. (Echiteae, Apocynaceae) in a montane rainforest in southern Ecuador.

PAs have been detected in several species of the related genus *Parsonsia* R. Br. (Edgar, 1984; Trigo et al., 1996b). Other genera of Apocynoideae reputed to contain PAs are *Alafia* Thouars, *Anodendron* A. DC., and *Pentalinon* Voigt (Hartmann & Witte, 1995). Due to lack of voucher specimens, however, the true identity of the plants analyzed is uncertain, diminishing the usefulness of these reports. Until additional tests show that these genera definitely contain PAs, it is more prudent to consider that in Apocynaceae they are restricted to Echiteae. Fifty-five valid species exist within the genus *Prestonia*; they are distributed in the New World between northern Argentina and Mexico (Morales, 2004). So far, PAs have been recorded in three species: *Prestonia acutifolia* (Benth. ex Müll. Arg.) K. Schum., *P. guatemalensis* Woodson, and *P. portobellensis* (Beurl.) Woodson (Edgar, 1984; Trigo & Brown, 1990; Trigo et al., 1996b; Pasteels et al., 2003). In samples of one additional species, *P. coalita* (Vell.) Woodson, no PAs could be detected (Trigo & Brown, 1990), but unconfirmed records suggest that plants in other populations of *P. coalita* contain PAs (Trigo et al., 1996b).

The New World Ithomiinae form a monophyletic clade with the Danainae and the Australasian genus *Tellervo* Kirby (Ehrlich, 1958; Ackery & Vane-Wright, 1984; Harvey, 1991; Brower, 2000; Freitas & Brown, 2004). Within this clade, the PAs are sequestered mostly by male butterflies and used as male pheromone precursors and in defense. Females are thought to obtain PAs with the spermatophore from males during mating (Brown, 1987). PAs provide a chemical defense against spiders such as *Nephila* Leach and other predators (Brown, 1984, 1987; Trigo et al., 1996a; Silva & Trigo, 2002; but see Collins & Watson, 1983; Pinheiro, 1996).

In addition to the ithomiine and danaine butterflies, the globally distributed arctiid moths are the other large group of Lepidoptera that has independently evolved the ability to sequester PAs for use as male pheromone precursors and chemical defense. Many arctiid moths sequester PAs from PA-containing host

plants as larvae (Weller et al., 1999), while others, especially in the Ctenuchini and Euchromiini, collect them as adults. PAs are transferred during mating to the females, and it has been shown that eggs of the arctiid moth *Utetheisa ornatrix* L. are efficiently protected against various egg predators and parasitoids (Eisner et al., 2002). Arctiid moths are generalists or host specialists, and they take up PAs either as larvae or as adults (or not at all, or as both; Boppré, 1990). Larval pharmacophagy has been demonstrated, for example, in *Cretonotos* Hübner (Boppré, 1984) and in *Estigmene acrea* Drury (Bernays et al., 2002; Singer et al., 2004), while adult pharmacophagy occurs in many arctiid genera (e.g., Pliske, 1975a; Goss, 1979).

Adult Lepidoptera use two principal natural sources of PAs in the Neotropical region: nectar and withered plant tissue of PA-containing plants. So far, the uptake of PAs with nectar has been observed only from certain plants of the Asteraceae (Eupatorieae) that often attract a specific guild of insects adapted to PAs (Arctiidae: *Senecio* L. (Jørgensen, 1913); Ithomiinae: *Adenostemma* J. R. Forst. & G. Forst., *Ageratum* L., *Emilia* Cass., *Eupatorium* L., and *Trichogonia* (DC.) Gardner (Moss, 1947; Beebe & Kenedy, 1957; Pliske, 1975b; Brown, 1987; Trigo et al., 1996b)). However, the presence of PAs in nectar has not been confirmed in most cases, and it is difficult to distinguish whether flowers are visited for nectar or PAs. PAs may also be obtained from withered or damaged tissue of PA-containing plants, most often on certain plants of the families Asteraceae (*Eupatorium*, *Senecio*), Boraginaceae (*Heliotropium* L., *Tournefortia* L.; Moss, 1947; Beebe, 1955; Pliske, 1975a, b; Brown, 1987), Fabaceae (*Crotalaria* L.), and with unspecified records on Apocynaceae (Brown, 1987). An exceptional case is provided by orchids of the genus *Epidendrum* L.; ithomiine butterflies are attracted by PAs and pollinate the plants, even though nectar is not provided (DeVries & Stiles, 1990).

MATERIAL AND METHODS

STUDY AREA AND FIELD TRIALS

Our first observations of ithomiine butterflies visiting withered Apocynaceae inflorescences were made on 5 and 12 December 1999 on the forest floor at Río Bombuscaro, near the entrance of Parque Nacional Podocarpus, south of Zamora, Province Zamora-Chinchipe, Ecuador, at ca. 1000 m a.s.l. (4°06.7'S, 78°58.8'W). More observations of ithomiines were made on 4 January 2000 (see Fig. 1), 7 and 27 October 2000, and on 2, 16, and 30 November 2000. *Prestonia* herbaria material (leaves and fresh



Figure 1. Two common male ithomiine visitors of withered inflorescences of *Prestonia amabilis* as a natural source of pyrrolizidine alkaloids (PAs) in a montane rainforest in southern Ecuador (1000 m a.s.l.), observed on 4 January 2000. Inflorescences fell from the ca. 18 m tall liana to the forest floor. —A. *Hypothyris euclea* subsp. *pyrippe* on a relatively fresh yellow inflorescence. —B. *Hyaliris mestra* subsp. *mestra* on an older blackish inflorescence.

inflorescences of the liana) was collected by F. Werner on 30 November 2000 at a height of ca. 12 m by accessing the canopy using the single-rope technique. The material was sent to a specialist who

later described the plant as a new species (Morales, 2004) and deposited the paratype specimen at INBio. Additional material was sampled for baiting experiments: withered inflorescences were collected from

Table 1. PA profiles established by gas chromatography–mass spectrometry for various organs of *Prestonia amabilis* and five visiting Lepidoptera specimens. All PAs represent retronecine esters (see Fig. 2). Itho 3 = *Napeogenes harbona*, undescribed subsp.; Itho 11 = *Hyalyris mestra* subsp. *mestra*; Itho 7 = *Hypothyris euclea* subsp. *pyrippe*; Cte 5 = *Leucopleura* sp. indet., cf. *caucadma*; M+ = molecular mass ion; m/z = mass/charge ratio; PA-X = unidentified pyrrolizidine alkaloid; tr = traces.

PAs recovered	m/z [M ⁺]	RI (DB-1 15 m)	Relative abundance (%)							
			<i>Prestonia amabilis</i>				5 Visiting Lepidoptera specimens			
			Roots	Leaves	Flowers	Itho 3	Itho 14	Itho 7	Cte 5	Cte 5
Retronecine	155	1422	8	15	14	tr	7	25	tr	100
PA-X	?	1655	3	3	2					
9-Angeloylretronecine	237	1801		12						
Supinine	283	1965					2			
Isoideamine A ¹	285	2048	4					20		
Ideamine A ^{1, 2}	285	2068	53	70	84	tr		55		
Indicine	299	2118				tr	58		tr	
Intermedine	299	2128				tr	21		tr	
Lycopsamine	299	2149					11			
3'-Acetylideamine ³	327	2185	17							
3'-Acetylideamine ³	327	2190	15							
3'-Acetylintermedine	341	2235					1			
Total alkaloids (µg/individual):						< 0.8	114	37	< 0.8	2.5
Total alkaloids (mg/g dry wt.):						< 0.1	6.3	6.7	< 0.1	0.4

¹ Tentatively identified; the precise assignment of possible diastereoisomers (see Fig. 2) was not possible.
² Occurrence as double peak indicating the presence of stereoisomers (identical mass fragmentation).
³ Representing two diastereoisomers (identical mass fragmentation).

the ground, and roots were dug up from the soil. Inflorescences and roots were subsequently dried in an oven at ca. 50°C. Roots were crushed in order to increase the release of secondary substances.

Field experiments were carried out between 29 October and 23 November 2000 at six locations in the area of the Reserva Biológica San Francisco (1800–2000 m a.s.l., 3°58.3'S, 79°04.7'W) (see Brehm et al., 2005 for a map) where *Prestonia amabilis* J. F. Morales is not known to occur. There was no precipitation in the period between 31 October and 15 November (the veranillo season), and there was moderate precipitation during the rest of November (G. Brehm, pers. obs.). At each location, two traps were installed close together (10–15 m), ca. 1 m above the ground. Depending upon the availability of plant material, between three and six pairs of traps were operated in parallel. The traps were designed according to DeVries (1987); they were 70 cm high and 40 cm in diameter. Approximately 1 g of PA-containing plant material, consisting of inflorescences or crushed root material, was used as bait in a plastic dish centered in the bottom of the trap. All traps were checked daily between 9:00 and 11:00 a.m., and fresh material (sampled 27 October, 2 and 16 November 2000) was provided every four to five days (inflorescences/root samples were regularly replaced by each other).

Arctiid moths were identified by comparison with type material in the Natural History Museum (London) by G. Brehm, and Ithomiinae butterflies were identified by K. Willmott. Lepidoptera voucher specimens are deposited in the Museum für Naturkunde, Stuttgart, Germany (SMNS). Statistical analyses were performed with the software Statistica 5.5 (StatSoft, Tulsa, Oklahoma).

PYRROLIZIDINE ALKALOID ANALYSIS

Plant materials included air-dried roots (1 g), leaves (0.6 g), and flowers (0.6 g) of *Prestonia amabilis*; these were available for PA identification and quantitative analysis. PAs were investigated in five Lepidoptera specimens (Table 1). Insects were freshly frozen and later spread and dried. It cannot be excluded that PAs might have been partly degraded during relaxation of the insects. Partially oxidized necine bases, generally a good indicator for postmortem degradations, could not be detected in any sample. The preparation of PA extracts from plant and insect samples, their subsequent separation and quantification by capillary gas chromatography, and their identification by combined gas chromatography–mass spectrometry was performed as described previously (Witte et al., 1993; Hartmann et al., 2005). The retention indices (RI) given in Table 1 refer to a 15 m DB-1 capillary column (J&W Scientific Inc., Folsom, California).

RESULTS

OBSERVATIONS IN NATURE

A total of four species of Ithomiinae were observed at withered inflorescences of *Prestonia amabilis* in the rainforest at 1000 m a.s.l. (Table 2). Figure 1 shows the two most common of these species (*Hypothyris euclea* subsp. *pyrippe* and *Hyalyris mestra* subsp. *mestra*) while taking up PAs from the withered plants through the proboscis. The inflorescences had a sweetish smell. Many butterflies (ca. 50) were observed on 5 and 12 December 1999 when ca. 100 withered inflorescences were present on the ground. Fewer butterfly individuals (ca. 2–20) were observed during subsequent visits to the site (January 2000 and October–November 2000), although many withered inflorescences were available (especially in November 2000). Ithomiine butterflies were also observed in the canopy overhead, but it is unknown whether they were actually visiting *P. amabilis*. A 20-cm-long section of *P. amabilis* was uprooted at 10:00 a.m. on 7 October 2000 and exposed. Six hours later, two males of *H. euclea* subsp. *pyrippe* were observed on this root. Only a single arctiid moth (*Pseudosphex* Hübner) was observed, during the day on 12 December 1999, sitting on withered inflorescences (D. Bartsch, pers. comm.). No other observations of arctiids (by day or at night) were made. However, two species, *Chetone histrio* Boisduval and *Sagaropsis* Hering, were found in the vegetation on 16 November 2000 within a 10-m radius, but it remains speculative whether or not they had visited *P. amabilis*.

FIELD TRIALS

The overall capture success of the baited traps was considered moderate. A total of 40 Ithomiinae (10 species) and 40 Arctiidae (8 species) were trapped (Table 2). Several moths and two butterflies escaped while the baits were emptied. By far, the most specimens and species were trapped at one site in a single pair of traps. Collecting data for each trap are available from the first author upon request. The highest numbers recorded during a single day were: Ithomiinae: 3 and 4 individuals (trap 9, 7 November; trap 3, 31 October); Arctiidae: 5 individuals (trap 1, 29 October; trap 2, 10 November). Because none, or only one or two individuals were trapped on most days, the results were summarized for the entire collecting period (Table 2). When all traps and species within the two clades are summarized, it becomes evident that the taxa show different preferences for either withered inflorescences (Ithomiinae) or crushed roots (Ctenuchini–Euchromiini; Table 2). This is confirmed by χ^2 tests of pooled samples

(Ithomiinae: $\chi^2 = 16.9$, $P < 0.001$; Ctenuchini–Euchromiini: $\chi^2 = 6.4$, $P < 0.05$, both $df = 1$). Because few individuals were collected in each species, the data do not allow a statistical analysis at species level.

Adult pharmacophagy has previously been observed in only five of the 13 Ithomiinae species recorded in the present study (Table 2). However, pharmacophagy had been reported in other studies for six out of seven genera. The only exception is the recently described genus *Megoleria* Constantino, containing two species, which is sister to *Hyposcada* Godman & Salvin (Willmott & Freitas, 2006). Uniquely within the Ithomiinae, *Megoleria* and *Hyposcada* larvae feed on Gesneriaceae (Drummond & Brown, 1987; A. V. L. Freitas & G. W. Beccaloni, pers. comm.; K. Willmott, pers. obs.), which are not known to contain PAs. In the Arctiidae, pharmacophagy has previously been reported in only one of the eight species listed (*Argyroides augiades* Druce; Table 2). So far, adult pharmacophagy has been recorded in three of the genera, but it has not previously been reported in three other genera (Table 2).

OTHER OBSERVATIONS

The species of Ithomiinae and Arctiidae listed in Tables 2 and 3 represent only a part of the local faunas of these taxa. Table 3 provides a list of 15 additional ithomiine species that were collected using an insect net in the study area in October and November 2000, while an additional 23 species have been recorded in this elevational range from the Zamora valley and could be present in the study area (K. Willmott and J. W. Hall, unpublished data). A list of 297 arctiid species recorded for the study area (1800–2005 m a.s.l.) is provided by Hilt and Fiedler (2006).

Pyrrolizidine alkaloids of Prestonia amabilis and selected insect visitors. Gas chromatography–mass spectrometry analysis of the plant samples revealed the presence of two novel PAs that were tentatively identified as 5'-demethyllycopsamine (ideamine A) and 5'-demethylisolycopsamine (isoideamine A; Fig. 2).

Ideamine A has not been previously found in plants, but it has been found in a danaine butterfly, *Idea leuconoe* Erichson, feeding on *Parsonia laevigata* (Moon) Alston (Apocynaceae; Nishida et al., 1991) and in the PA-sequestering Neotropical leaf beetle *Platyphora boucardi* Jacoby, feeding on *Prestonia portobellensis* (Hartmann et al., 2003). In both insects, ideamine A was found to be an insect transformation product of the sequestered macrocyclic

Table 2. Pharmacophagy of 13 Ithomiinae butterflies and eight Arctiidae (Ctenuchini–Euchromiini) moths observed on (1) withered inflorescences and (2) crushed roots of *Prestonia amabilis*. Lepidopterans were attracted using baited traps in a montane rainforest in southern Ecuador (1800–2000 m a.s.l.). All Ithomiinae and most Arctiidae specimens were males. Females were trapped in *Leucopleura* sp. (1 out of 10), *Cyanopepla imperialis* (2 out of 6), and *Argyroeides augiades* (4 out of 5). Pharmacophagy toward PAs has previously been observed for five of the 13 Ithomiinae species listed (Beebe, 1955; Pliske, 1975b; Edgar & Pliske, 1976; Brown, 1985, 1987). PA pharmacophagy has previously been reported for only one of the listed Arctiidae species and has so far not been recorded in the genera *Leucopleura*, *Merania*, and *Microgiton* (Jørgensen, 1913; Moss, 1947; Beebe & Kenedy, 1957; Pliske, 1975b; Goss, 1979; Häuser & Boppré, 1997). Records identified as “new” are at a global scale. d/n = diurnal/nocturnal behavior was observed (nocturnal observations: Hilt & Fiedler, 2005); qual = qualitative observations of pharmacophagy on withered flowers made at the original collecting site of *P. amabilis* (ca. 1000 m a.s.l.); KW = K. Willmott; MS = manuscript in preparation.

ID	Species	Inflorescence trap no.							Root trap no.							d/n	Pharmacophagy
		1	3	5	7	9	11	sum	2	4	6	8	10	12	sum		
	Ithomiinae																
8	<i>Greta alphasiboea</i> Hewitson, 1869	-	-	-	-	-	-	0	-	-	-	-	1	-	1	d	New record for species
4	<i>Greta andromica</i> subsp. <i>andania</i> Hopffer, 1874	-	-	-	-	1	-	1	-	-	-	-	-	-	0	d	KW, pers. obs., Ecuador
10	<i>Greta ortygia</i> subsp. <i>ortygia</i> Weymer, 1890	-	-	-	-	-	1	1	-	-	-	-	2	-	2	d	KW, pers. obs., Ecuador
1	<i>Hyalyris antea</i> subsp. <i>amarilla</i> Vitale & Bollino, 2000	3	1	-	-	-	-	4	-	-	-	-	-	-	0	d	New record for species
2	<i>Hyalyris praxilla</i> , undescribed subsp. Willmott & Lamas	5	1	-	-	-	-	6	-	-	-	-	-	-	0	d	New record for species
14	<i>Hyalyris mestra</i> subsp. <i>mestra</i> Hopffer, 1874							qual								d	New record for species
7	<i>Hypothyris euclea</i> subsp. <i>pyrippe</i> Hopffer, 1874	-	-	-	-	-	1	qual	-	-	-	-	-	-	0	d	Known from other subsp.
12	<i>Hypothyris mansuetus</i> subsp. <i>amica</i> Weymer, 1883							qual								d	New record for species
11	<i>Ithomia agnosia</i> subsp. <i>agnosia</i> Hewitson, 1855							qual								d	KW, pers. obs., Ecuador
6	<i>Megoleria orestilla</i> subsp. <i>orestilla</i> Hewitson, 1867	-	-	-	-	-	-	0	-	-	1	-	1	-	2	d	New record for genus
3	<i>Napeogenes harbona</i> , undescribed subsp. Vitale & Willmott	4	1	1	-	2	-	8	1	-	-	-	1	-	2	d	New record for species
9	<i>Napeogenes lycora</i> , undescribed subsp. Vitale & Willmott, MS	1	-	-	1	-	-	2	-	-	-	-	-	-	0	d	New record for species
5	<i>Oleria athalina</i> subsp. <i>banjana</i> Haensch, 1903	3	-	1	-	4	2	10	-	-	-	-	-	-	0	d	KW, pers. obs., Ecuador
	Sum	16	3	2	1	7	4	33	1	-	1	-	5	-	7	d	New record for species
	Arctiidae																
3	<i>Argyroeides augiades</i> Druce, 1896	2	-	-	-	-	-	2	3	-	-	-	-	-	3	d/n	Known from species
6	<i>Cyanopepla argyrtidia</i> Hampson, 1898	-	-	-	-	-	-	0	3	-	-	-	-	-	3	d	New record for species; known from genus
1	<i>Cyanopepla imperialis</i> Druce, 1883	1	-	-	-	1	-	2	3	-	-	-	1	-	4	d	New record for species; known from genus
7	<i>Euagra chica</i> Hampson, 1898	1	-	-	-	-	1	2	2	-	-	1	-	-	3	d/n	New record for species; known from genus
5	<i>Leucopleura</i> sp. indet., cf. <i>caucadma</i> Druce, 1894	3	-	-	-	-	1	4	4	1	-	-	1	-	6	d/n	New record for genus
2	<i>Mevania basalis</i> (?) Walker, 1864	-	-	-	-	-	-	0	1	-	-	-	-	-	1		New record for genus
3	<i>Microgiton submacula</i> Walker, 1854	-	-	-	-	-	-	0	-	-	-	1	-	-	1		New record for genus
4	<i>Sphaecosoma</i> sp.	2	-	-	-	-	-	2	4	1	-	-	2	-	7	d	Known from genus
	Sum	9	-	-	-	1	2	12	20	2	-	2	4	-	28		

Table 3. Other Ithomiinae species, collected by G. Brehm in the study area in southern Ecuador using an insect net, that were not observed at *Prestonia amabilis* baits. All species that were trapped at *P. amabilis* bait stations (Table 2) were also collected with nets (with the exception of *Megoleria orestilla* subsp. *orestilla*), but are not listed here. Note that butterflies were not sampled quantitatively. However, the numbers suggest a rough estimate of abundance in the area. KW = Keith Willmott; MS = manuscript in preparation.

ID	Species	Individuals collected	Pharmacophagy
10	<i>Dircenna adina</i> subsp. <i>lorica</i> Weymer, 1875	3	KW, pers. obs., Ecuador
6	<i>Episcada mira</i> Hewitson, 1877	2	KW, pers. obs., Ecuador
14	<i>Godyris dircenna</i> C. Felder & R. Felder, 1865	1	A. V. L. Freitas, pers. comm.
12	<i>Godyris hewitsoni</i> , undescribed subsp. Willmott, MS	2	Records for genus
13	<i>Greta dercetis</i> subsp. <i>dercetis</i> Doubleday, 1847	1	Record for species (Edgar & Pliske, 1976)
16, 17	<i>Ithomia avella</i> subsp. <i>epona</i> Hewitson, 1869	1	KW, pers. obs., Ecuador
18	<i>Ithomia cleora</i> Hewitson, 1855	2	KW, pers. obs., Ecuador
19	<i>Ithomia salapia</i> subsp. <i>derasa</i> Hewitson, 1855	2	KW, pers. obs., Ecuador
3	<i>Ithomia terra</i> subsp. <i>terra</i> Hewitson, 1853	6	KW, pers. obs., Ecuador
9	<i>Napeogenes flossina</i> , undescribed subsp. Vitale & Willmott, MS	3	On Asteraceae (KW, pers. obs., Ecuador)
11	<i>Oleria estella</i> subsp. <i>estella</i> Hewitson, 1868	1	Records for genus
5	<i>Oleria makrena</i> subsp. <i>makrenita</i> Haensch, 1903	3	KW, pers. obs., Ecuador
20	<i>Patricia oligyrtis</i> subsp. <i>oligyrtis</i> Hewitson, 1877	4	<i>Patricia dercyllidas</i> observed on Asteraceae flowers and tree (KW, pers. obs., Ecuador)
7	<i>Pteronymia alissa</i> subsp. <i>andreas</i> A. G. Weeks, 1901	6	KW, pers. obs., Ecuador
15	<i>Pteronymia zerlina</i> subsp. <i>machay</i> T. & L. Racheli, 2003	10	KW, pers. obs., Ecuador

triester alkaloid 14-deoxyparsonsianidine. The stereochemistry of the necic acid moiety at the 2' and 3' carbons remains obscure. With all plant samples, the gas chromatography profiles show ideamine A as

double peak with identical mass fragmentation patterns indicating the presence of at least two stereoisomers. This is also indicated by the presence of two isomeric 3'-acetyl esters of ideamine A in the

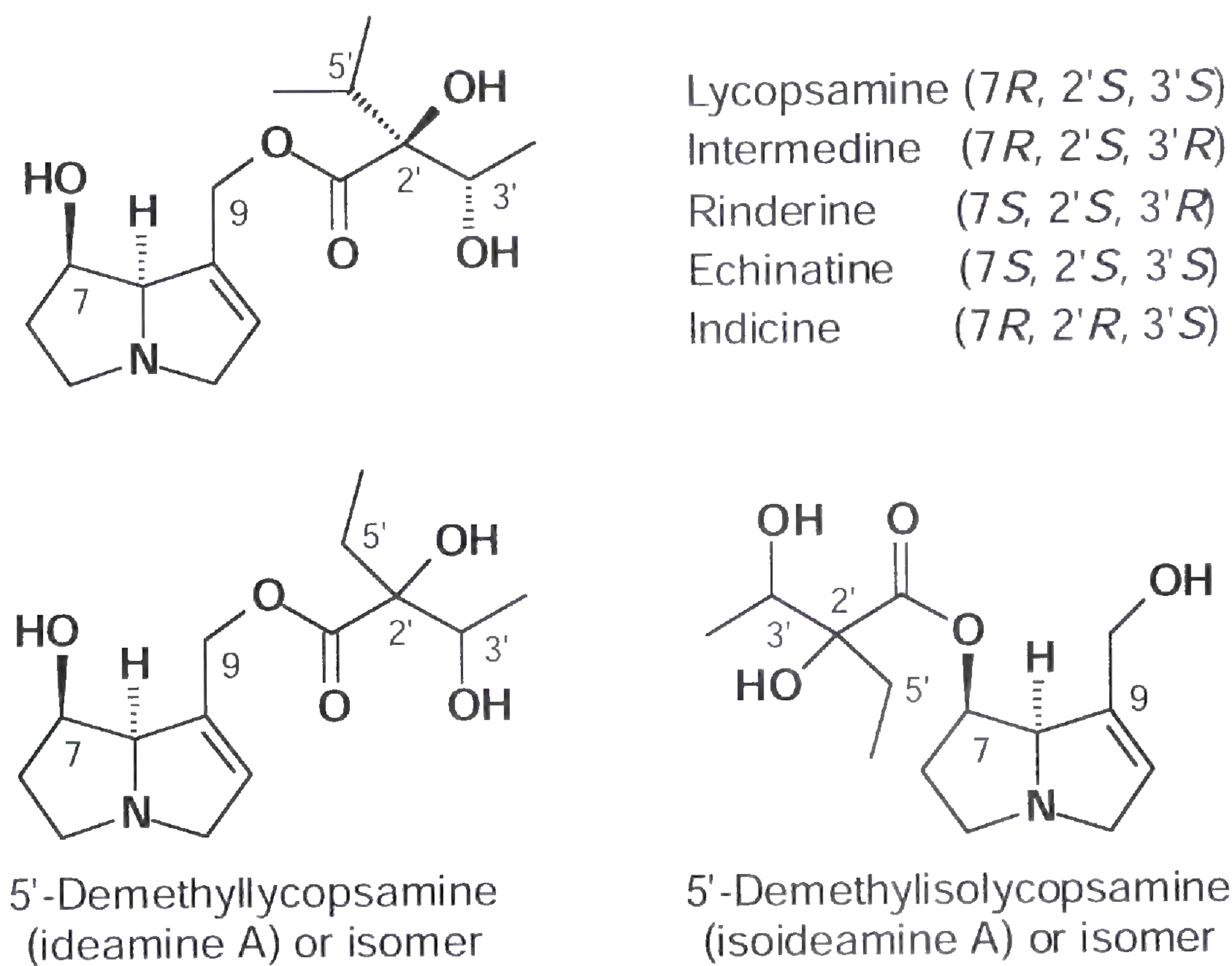


Figure 2. Top: Pyrrolizidine alkaloids of the lycopamine type showing the unique branched-chain C₇ necic acids esterified with retronecine (*7R*) or heliotridine (*7S*). Bottom: The two novel structures identified from *Prestonia amabilis*.

root samples (Table 1). All PAs identified from the plant materials are esters of the necine base retro-necine (Table 1).

The two stereoisomers of ideamine A always represent the majority of PAs, ranging from 53% (roots) to 84% of total PAs in flowers (Table 1). Inflorescences display the highest PA concentration (ca. 0.13% alkaloid per dry matter), followed by roots and leaves (Table 1).

Among the five insect samples analyzed, two showed clear plant-derived PA profiles (Table 1). The specimen of *Hypothyris euclea* subsp. *pyrippe* shows the *Prestonia amabilis* pattern, indicating that it obtained its PAs pharmacophagously from this plant species. The specimen of *Hyaliris mestra* subsp. *mestra* did not show the *P. amabilis* PA pattern, but instead contained lycopsamine and two of its isomers (Fig. 2), indicating that it previously gained PAs from the Boraginaceae (e.g., *Heliotropium*) or an Asteraceae (Eupatorieae). The other three specimens, belonging to an undescribed subspecies of *Napeogenes harbora* and to *Leucopleura*, showed only trace amounts of PAs, without a profile indicative for a specific PA-containing host plant.

DISCUSSION

Most ithomiine butterflies are apparently adapted to PAs of the lycopsamine type. These PAs are characterized by their unique branched-chain C₇ necic acid moiety (Fig. 2; Hartmann & Ober, 2000; Weber et al., 1999). The occurrence of PAs of the lycopsamine type is restricted to certain species of three plant families: the Boraginaceae, the Asteraceae (only Eupatorieae), and the Apocynaceae (Hartmann & Witte, 1995). PAs of the lycopsamine type occur mostly as monoesters (Fig. 2), but occasionally also occur as open-chain diesters or macrocyclic triesters. The latter are only known from certain species of the Apocynaceae (e.g., *Parsonsia*). There are several lines of evidence indicating a specific preference of ithomiine butterflies for PAs of the lycopsamine type: (1) PAs recovered from field-caught butterflies are almost exclusively monoesters of the lycopsamine type (Brown, 1984, 1987; Trigo et al., 1996a); (2) the butterflies specifically transform the various diastereoisomers (see Fig. 2) into lycopsamine and intermedine, which represent the major PAs recovered from ithomiines (Trigo et al., 1994, 1996a); (3) feeding experiments revealed that other monoesters (e.g., seneciylretronecine) and macrocyclic PAs of the senecionine type are less efficiently sequestered and are not stably retained (Brückmann et al., 2000); (4) most species convert the specific C₇ necic acid of lycopsamine PAs into male pheromones (i.e., itho-

mine γ -lactones), which are released from the male hairpencils (Schulz, 1998; Schulz et al., 2004). In this context, it is remarkable that *Prestonia amabilis* appears to be attractive for ithomiines, although it contains the two novel PAs, ideamine A and isoideamine A, which, instead of the unique C₇ necic acid, possess the respective lower homologues with one methyl group less (Fig. 2). Because neither lycopsamine nor one of its diastereoisomers could be detected in *P. amabilis*, ideamine A must be responsible for the attractiveness of the plant to ithomiines.

Withered inflorescences of *Prestonia amabilis* are apparently a natural source of PAs for Ithomiinae and Arctiidae in our study area, although only relatively few species (four of Ithomiinae and one of Arctiidae) were actually observed feeding in a natural situation. Our study demonstrates for the first time unambiguous pharmacophagy by Lepidoptera on *Prestonia*. The only previous hint was provided by Trigo et al. (1996b), who reported visits of two males and one female of the primitive ithomiine *Tithorea harmonia* on flowers of *Prestonia acutifolia*. The fact that pharmacophagy was observed for the first time for one ithomiine and three arctiid genera, as well as for most species (8/13 Ithomiinae species, 7/8 arctiid species), suggests that many more such relationships are likely to exist in Neotropical rainforests (see also Häuser & Boppré, 1997).

Observations of unmanipulated pharmacophagous behavior are generally scarce in the Neotropical region. Pharmacophagy has hardly been reported from regions such as the Andean montane forests in Peru and Ecuador, where the greatest diversity of Ithomiinae and Arctiidae occurs. Most previous observations have been made with uprooted plants, especially by using the highly attractive *Heliotropium indicum* L., other species of *Heliotropium*, and *Eupatorium* species (Moss, 1947; Beebe, 1955; Beebe & Kenedy, 1957; Pliske, 1975a; Goss, 1979; Häuser & Boppré, 1997). These experimental manipulations give important insights into the behavior of the insects. However, little is still known about the butterflies' and moths' preferences for different PA resources in their habitats. Brown (1987) provided a list of Ithomiinae genera that visited either withered Boraginaceae plants or withered plants plus nectar of Eupatorieae (Asteraceae). Species within most Ithomiinae genera were strongly attracted to both sources, but females were obviously less attracted to decomposing plants and more often attracted to the Eupatorieae flowers. This finding is supported by our study, in which exclusively males of Ithomiinae were collected at the *Prestonia* PA sources (that provide no nectar). However, Brown (1987) also showed that not all

Table 4. Comparison of potential and actual numbers of Ithomiinae recorded in pharmacophagy in the study area in southern Ecuador. Potential numbers are taxa recorded from Río Bombuscaro and in the province of Zamora-Chinchipe between 1800–2100 m by K. Willmott and J. W. Hall (unpublished data) during four months of field work over a 10-year period (1993–2003).

Tribe	Potential species	Recorded pharmacophagous species
New tribe (<i>Athesis</i> – <i>Patricia</i>)	2	0
Dircennini	19	0
Godyridini	15	3
Ithomiini	5	1
Mechanitini	1	0
Melinaeini	1	0
Napeogenini	10	7
Oleriini	10	2
Total	63	13

genera were equally attracted; a weak response was recorded in some notably primitive (Willmott & Freitas, 2006) genera (*Tithorea* and *Methona*), perhaps because they already sequester PAs from their host plants.

The lists provided in Tables 2 and 3 represent incomplete samples of pharmacophagous Ithomiinae and Arctiidae of the study area; a longer sampling period and broader elevational range would result in additional records of rare species. Nevertheless, some phylogenetic patterns are apparent. For example, *Ithomia terra* subsp. *terra*, *Pteronymia alissa* subsp. *andreas*, and *P. zerlina* subsp. *machay* were all relatively abundant, with six individuals of each species recorded, but none of these species were recorded at baits. Notably, none of the 19 species of ithomiine tribe Dircennini potentially present at the study sites was observed in pharmacophagy, and only three of the 15 species of Godyridini (Table 4), of which *Greta andromica* and *G. ortygia* are two of the most abundant montane ithomiine species, were observed in pharmacophagy. Males of Dircennini and Godyridini are, however, frequently observed feeding at Asteraceae flowers (e.g., Table 3), and this sex-specific behavior strongly suggests they are seeking PAs. It may also be significant that, although the Napeogenini comprise only 10 of the 63 potential species in the study area (16%), seven of the 13 species observed in pharmacophagy are Napeogenini, suggesting a particularly strong attraction within this group of species to *Prestonia*. Napeogenini are characteristic of deep shade, where *Prestonia* occurs, whereas Dircennini and Godyridini are often observed

in the forest canopy and at forest edges (K. Willmott & G. Brehm, pers. obs.), where Asteraceae PA-containing flowers grow; this suggests the habitat differences may play a role in preferred PA sources. Elsewhere in Ecuador, we also observed males of three ithomiine species, *Patricia derycyllidas* subsp. *derycyllidas* (undescribed tribe), *Napeogenes larilla* (undescribed subspecies), and *N. flossina* (undescribed subspecies; Napeogenini), feeding on stems and leaves of an apparently undamaged, unidentified Asteraceae tree in cloud forest at 2400 m a.s.l. (K. Willmott, pers. obs.). Deeper forest ithomiines may therefore use a variety of PA sources (e.g., *Prestonia*) about which we know comparatively little.

Among the arctiids, only eight species were recorded at baits. This is a small proportion compared with the many Arctiidae species collected at light traps in the study area. Between 1800 and 2005 m, a total of 297 nocturnal Arctiidae species were recorded (among them 82 Ctenuchini–Euchromiini species; Hilt & Fiedler, 2006). However, only three of the species collected in the *Prestonia* traps were also recorded in these nightly samples (Table 2). Six species found in the traps were also observed flying by day. Previous studies (hand-sampling) recorded a higher number of nocturnal arctiid moths at PA baits than observed in our study (e.g., Beebe, 1955; Häuser & Boppré, 1997). A modification of the trap design (e.g., toward a funnel-design) could possibly increase the number of collected individuals.

Prestonia may have been overlooked previously as a natural source of PA for adult Lepidoptera because the genus consists of vines and lianas. It is more difficult to observe Lepidoptera behavior at plants with this habit than at herbs such as *Heliotropium* or *Eupatorium*. PA-containing Apocynaceae host plants are a key for the understanding of the evolution of PA pharmacophagy in the closely related Danainae, Ithomiinae, and *Tellervo* (Edgar, 1984; Trigo et al., 1996b). Although caterpillars of ca. 95% of ithomiine species feed only on Solanaceae, the likely ancestral host plant family is Apocynaceae, which is found as a host in *Tellervo* and which is a widespread host in the Danainae. Caterpillars of the primitive ithomiine genera *Tithorea*, *Elzunia*, and *Aeria* also use *Prestonia* species as host plants (Brown, 1987; DeVries, 1987; Drummond & Brown, 1987; Trigo & Brown, 1990). Virtually all of the more derived ithomiine species have the ability to sequester PAs and take them up as adults from various plant sources (Brown, 1987; Schulz et al., 2004; Trigo et al., 1996b). It seems likely that a direct ancestor of *Prestonia* may have served as the original additional source of alkaloids for the adults of these species.

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